

# Steam cooking significantly improves in vitro bile acid binding of beets, eggplant, asparagus, carrots, green beans, and cauliflower<sup>☆</sup>

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## Abstract

Cholesterol-lowering potential of foods and food fractions have been evaluated by determining their bile acid-binding potential. Reducing bile acid recirculation lowers cholesterol by reducing fat absorption and use of cholesterol to synthesize bile acid. Secondary bile acids increase the risk of cancer. Bile acid-binding potential is related to lowering the risk of heart disease as well as cancer prevention. Previously, we have reported bile acid binding by several uncooked vegetables. However, most vegetables are consumed after cooking. How cooking would influence in vitro bile acid binding of various vegetables was investigated using a mixture of bile acids secreted in human bile under physiologic conditions. Eight replicate incubations were conducted for each treatment simulating gastric and intestinal digestion, which included a substrate only, a bile acid mixture only, and 6 with a substrate and bile acid mixture. Cholestyramine (a cholesterol-lowering, bile acid-binding drug) was the positive control treatment, and cellulose was the negative control. Relative to cholestyramine, in vitro bile acid binding on a dry matter basis was, for beets, 18%; okra, 16%; eggplant, 14%; asparagus, 10%; carrots, 8%; green beans, 7%; cauliflower, 6%; and turnips, 1%. These results point to the significantly different ( $P \leq .05$ ) health-promoting potential of these vegetables (from highest to lowest, beets, okra, eggplant, asparagus, carrots and green beans, cauliflower, turnips) as indicated by their bile acid binding on a dry matter basis. Steam cooking significantly improved in vitro bile acid binding of beets, eggplant, asparagus, carrots, green beans, and cauliflower compared with previously observed bile acid-binding values for these vegetables uncooked. Inclusion of steam-cooked beets, okra, eggplant, asparagus, carrots, green beans, and cauliflower in our daily diet as health-promoting vegetables should be encouraged. These vegetables, when consumed regularly, may lower the risk of premature degenerative diseases (heart disease and cancer), improve public health, and advance human nutrition research.

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**Keywords:** In vitro; Bile acid binding; Beets; Okra; Eggplant; Asparagus; Carrots; Green beans; Cauliflower; Turnips

## 1. Introduction

The United States Department of Agriculture (USDA) Food and Nutrition Information Center's Food Guide Pyramid—Steps to a Healthier You (<http://www.mypyramid.gov>).

<sup>☆</sup> The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

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[www.mypyramid.gov](http://www.mypyramid.gov)) recommends the consumption of colorful vegetables and low-fat food products along with daily active life and maintenance of desirable body weight [1]. Some of the vegetables listed by the USDA food pyramid include asparagus, beets, carrots, cauliflower, eggplant, green beans, okra, and turnips. Vegetarians or those consuming vegetables as a major portion of their daily diet along with less energy from saturated fat and animal products are at a lower risk of coronary heart disease and cancer. Vegetables are a good source of dietary fiber, antioxidants, phytonutrients, provitamins,

polyphenols, and minerals. Antioxidants in beets and green beans [2,3], probiotics and immune-protecting phytochemicals of asparagus [4–6], hydroxycinnamic acid of eggplant [7], and glucosinolates of cauliflower [8,9] have been associated with health-promoting effects. Phytonutrients in the vegetables have been shown to stimulate natural detoxifying enzymes in the body and lower the risk of atherosclerosis and cancer [10,11]. Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer [12]. The healthful, cholesterol-lowering (atherosclerosis amelioration) or detoxification-of-harmful-metabolites (cancer prevention) potential of food fractions could be predicted by evaluating their in vitro bile acid binding, based on positive correlations found between in vitro and in vivo studies showing that cholestyramine (bile acid-binding, cholesterol-lowering drug) binds bile acids, whereas cellulose does not show this effect [13–16]. Atherosclerosis and cancer are major public health problems in the developed world and are becoming prevalent in the emerging world with greater affluence because of outsourcing of jobs and globalizing of industrial production facilities to the lower-cost-labor countries. To lower the risk of diet- and lifestyle-related premature degenerative diseases and to advance human nutrition research, relative bile acid-binding potential of foods and fractions need to be evaluated. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation [17]. Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism by which food fractions lower cholesterol [18–20]. The bile acids are needed for the absorption of dietary fat from the gastrointestinal tract. The dietary fat is a precursor of cholesterol synthesis in the body. By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids [21–24]. Excretion of toxic metabolites and secondary bile acids could lower the risk of cancer [12]. Bile acid binding of grain fractions, ready-to-eat cereals, and various dry beans has been observed to be proportional to their dry matter (DM) content [25–28]. The in vitro bile acid binding of uncooked vegetables on a DM basis has been reported relative to cholestyramine: for okra, 16%; beets, 11%; asparagus, 4%; and 1% for eggplant, turnips, green beans, carrots, and cauliflower [29]. Vegetables are normally cooked before consumption; how cooking would influence their bile acid binding has not been previously reported.

The objective of this study was to evaluate healthful potential of steam-cooked beets (*Beta vulgaris*), okra (*Abelmoschus esculentus*), eggplant (*Solanum malongena*), asparagus (*Asparagus officinalis*), carrots (*Daucus carota*), green beans (*Phaseolus vulgaris*), cauliflower (*Brassica oleracea botrytis*), and turnips (*Brassica rapa rapifera*), as determined by their bile acid binding on an equal DM basis, with a bile acid mixture observed in human bile under duodenal physiologic pH of 6.3.

## 2. Methods and materials

Fresh beets, okra, eggplant, asparagus, carrots, green beans, cauliflower, and turnips were obtained from a local grocery supermarket. All the vegetables were washed, cut into bite-size pieces, and steam cooked in a double boiler to a ready-to-be-eaten tenderness; steaming cooking time for beets, okra, and green beans was 15 minutes; for eggplant, asparagus, carrots, cauliflower, and turnips, 10 minutes (Table 1). All the cooked vegetables were dried to a constant weight at 68°C for 48 hours in a steam-heated forced-air food dryer (Proctor 062; Proctor and Schwartz, Inc, Horsham, Pa). Dry samples were ground in a Thomas-Wiley Mini Mill (Arthur Thomas, Philadelphia, Pa) to pass a 0.4-mm screen. Total dietary fiber (TDF) of the test samples was determined by method 985.29 [30]. A brief description of the TDF method is as follows: Duplicate test portions of dried foods (fat-extracted if containing >10% fat) were gelatinized with Termamyl (heat-stable  $\alpha$ -amylase) and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol were added to precipitate soluble dietary fiber. Total residue was filtered and washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue was weighed. One duplicate was analyzed for protein, another was incinerated at 525°C, and ash was determined. Total dietary fiber = weight residue – weight (protein + ash).

Samples were analyzed for nitrogen by method 990.03 [31], with a Virio Macro Elemental Analyser (Elementar Analysen systeme GmbH, Hanau, Germany), crude fat with petroleum ether by an accelerated solvent extractor (ASE 200 Dionex Corp, Sunnyvale, Calif), ash by method 942.05 [31], and moisture by method 935.29 [31]. Cholestyramine, a bile acid-binding anionic resin (a drug that lowers cholesterol and binding bile acids), was the positive control treatment; cellulose (a non-bile acid-binding fiber) was the negative control. Both were obtained from (Sigma, St Louis, Mo).

Table 1  
Dry matter content of steam-cooked beets (*B vulgaris*), okra (*A esculentus*), eggplant (*S malongena*), asparagus (*A officinalis*), carrots (*D carota*), green beans (*P vulgaris*), cauliflower (*B oleracea botrytis*), and turnips (*B rapa rapifera*)

Source	Cut to size <sup>a</sup>	Steamed (min)	DM (%)
Beets	0.5- to 0.75-in cubes	15	10.03
Okra	0.5- to 0.75-in pieces	15	8.84
Eggplant	0.5- to 0.75-in cubes	10	7.07
Asparagus	1.0- to 2.0-in pieces	10	5.99
Carrots	0.5- to 0.75-in pieces	10	13.84
Green beans	1.0- to 1.5-in pieces	15	8.63
Cauliflower	1.0- to 2.0-in florets	10	7.33
Turnips	0.5- to 0.75-in cubes	10	9.95

<sup>a</sup> Fresh vegetables (400–500 g) were washed, trimmed, and cut into bite-size pieces and steamed in a double boiler; DM was determined after drying at 68°C for 48 hours in a food dehydrator.

### 2.1. Bile acid-binding procedure

The in vitro bile acid-binding procedure was a modification of that by Camire et al [32], as previously reported [26]. The stock bile acid mixture was formulated, with glycocholic bile acids providing 75% and taurine-conjugated bile acids 25% of the bile acids, based on the composition of the human bile [33,34]. This mixture contained glycocholic acid (9 mmol/L), glycochenocholic acid (9 mmol/L), glycodeoxycholic acid (9 mmol/L), taurocholic acid (3 mmol/L), taurochenocholic acid (3 mmol/L), and taurodeoxycholic acid (3 mmol/L) in 0.1 mol/L phosphate buffer with pH 6.3. This stock solution of 36 mmol/L was stored at  $-20^{\circ}\text{C}$  and diluted to the working solution (0.72  $\mu\text{mol/mL}$ ) just before each assay. Eight replicate incubations—6 substrates with bile acid mixture, 1 substrate blank without bile acid mixture, and 1 bile acid mixture without substrate—were run for each of the 8 vegetables and 2 control treatments. Each treatment replicate was weighed into a  $16 \times 150$ -mm glass screw-capped tube. Samples were digested in 1 mL 0.01N hydrochloric acid (HCl) for 1 hour in a  $37^{\circ}\text{C}$  shaker bath. After this acidic incubation, which simulated gastric digestion, the sample pH was adjusted to 6.3 with 0.1 mL of 0.1N sodium hydroxide (NaOH). To each test sample was added 4 mL of bile acid mixture working solution. A phosphate buffer (4 mL, 0.1 mol/L, pH 6.3) was added to the individual substrate blanks. After the addition of 5 mL of porcine pancreatin (5X, 10 mg/mL, in a 0.1-mol/L phosphate buffer, pH 6.3, providing amylase, protease, and lipase for digestion of samples), tubes were incubated for 1 hour in a  $37^{\circ}\text{C}$  shaker bath. Mixtures were transferred to 10-mL centrifuge tubes (Oak Ridge 3118-0010; Nalgene, Rochester, NY) and centrifuged at 99000g in a 75 Ti rotor at 39 K for 18 minutes at  $25^{\circ}\text{C}$  in an ultracentrifuge (model L-60; Beckman, Palo Alto, Calif). The supernatant was removed into a second set of labeled tubes. An additional 5 mL of phosphate buffer was used to rinse out the incubation tube and added to the centrifuge tube, which was vortexed and centrifuged as before. The supernatant was removed and

combined with the previous supernatant. Aliquots of pooled supernatant were frozen at  $-20^{\circ}\text{C}$  for bile acid analysis. Bile acids were analyzed by the Trinity Biotech bile acids procedure number 450-A (Trinity Biotech Distribution, St Louis, Mo) using a Ciba-Corning Express Plus analyzer (Polestar Labs, Inc, Escondido, Calif). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing Trinity Biotech bile acid calibrators (no 450-11) at 5, 25, 50, 100, and 200  $\mu\text{mol/L}$ . Individual blank substrates were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank).

### 2.2. Statistical analysis

Data are presented as means  $\pm$  SEM. Before accepting analysis of variance results, Lavene test was used to check for homogeneity of variances among treatments. Because variances were considered homogenous from test results, analysis of variance was used to test for significant differences among treatments. Dunnett 1-tailed test was computed with treatments compared with cholestyramine as a positive control and compared with cellulose as a negative control. For comparing noncontrol treatments with each other, Tukey test for comparison of all possible pairs of means was used. SAS Proc GLM (SAS Institute, Cary, NC) was used for all statistical analysis and testing [35,36]. A value of  $P \leq .05$  was considered the criterion of significance.

## 3. Results

The bite-size cut pieces, steam cooking time, and DM content of vegetables as ready to be eaten is given in Table 1. Dry matter content of the steam-cooked vegetables was highest for carrots 14% and lowest for asparagus 6.0%. Composition of the beets, okra, eggplant, asparagus, carrots, green beans, cauliflower, and turnips is given in Table 2. Both cellulose and cholestyramine were considered as 100% TDF. There was wide variation in the dietary fiber and protein

Table 2

Composition of steam-cooked [and raw<sup>a</sup>] beets (*B vulgaris*), okra (*A esculentus*), eggplant (*S malongena*), asparagus (*A officinalis*), carrots (*D carota*), green beans (*P vulgaris*), cauliflower (*B oleracea botrytis*), and turnips (*B rapa rapifera*), on a DM basis

Source	TDF <sup>b</sup> (DM %)	Protein <sup>c</sup> (DM %)	Fat <sup>c</sup> (DM %)	Minerals <sup>c</sup> (DM %)	Carbohydrates <sup>d</sup> (DM %)
Beets	23.8 [23]	17.7 [13]	0.4 [1]	6.3 [9]	76.0 [77]
Okra	43.8 [33]	25.3 [20]	0.5 [1]	8.3 [7]	66.4 [72]
Eggplant	37.6 [45]	15.6 [13]	0.3 [3]	6.7 [9]	77.6 [75]
Asparagus	28.7 [31]	32.2 [32]	1.6 [2]	8.6 [9]	59.2 [57]
Carrots	29.8 [24]	9.6 [8]	1.1 [2]	7.9 [8]	82.5 [82]
Green beans	31.7 [35]	22.1 [19]	0.9 [1]	6.8 [7]	71.1 [73]
Cauliflower	28.4 [31]	27.8 [25]	1.7 [1]	10.1 [9]	62.1 [66]
Turnips	29.2 [22]	13.6 [11]	0.2 [1]	8.5 [9]	77.9 [79]
Cholestyramine	100.0	—	—	—	—
Cellulose	100.0	—	—	—	—

<sup>a</sup> Data from Kahlon et al [29]. Raw vegetables were obtained from the same local grocery supermarket.

<sup>b</sup> n = 6.

<sup>c</sup> n = 3.

<sup>d</sup> Carbohydrates = [100 - (protein + crude fat + ash)].

content of these vegetables. Total dietary fiber and protein values, respectively, on a DM basis for the steam-cooked vegetables were as follows: beets, 24% and 18%; okra, 44% and 25%; eggplant, 38% and 16%; asparagus, 29% and 32%; carrots, 30% and 10%; green beans, 32% and 22%; cauliflower, 28% and 28%; and turnips, 29% and 14%. On a DM basis, fat and mineral content in the cooked vegetables tested were 0.2% to 1.7% and 6% to 10%, respectively. The values for raw vegetables obtained from the same grocery store are shown in brackets [29].

On an equal DM basis, bile acid binding was significantly higher for cholestyramine than all the steam-cooked vegetables tested (Table 3). There were significant ( $P \leq .05$ ) differences in the bile acid binding between all the cooked vegetables tested except for carrots and green beans, whose values were similar. The highest bile acid binding was observed for beets, and the lowest values were for turnips. Assigning a bile acid-binding value of 100% to cholestyramine, the relative bile acid binding on a DM basis for the test samples of steam-cooked vegetables was as follows: beets, 18%; okra, 16%; eggplant, 14%; asparagus, 10%; carrots, 8%; green beans, 7%; cauliflower, 6%; and turnips, 1%. Relative bile acid binding on a DM basis was, from highest to lowest, as follows: beets, okra, eggplant, asparagus, carrots and green beans, cauliflower, turnips.

The bile acid binding on equal TDF basis is shown in Table 4. Cholestyramine bound bile acids significantly more than the various steam-cooked vegetables tested. On a TDF basis, considering cholestyramine as 100% bound, bile acid-binding values were as follows: beets, 73%; okra, 37%; eggplant, 37%; asparagus, 34%; carrots, 25%; green beans, 23%; cauliflower, 22%; and turnips, 4%. Bile acid-binding values on a TDF basis among various steam-cooked vegetables tested were, from highest to lowest, as follows: beets, okra and eggplant, asparagus, carrots, green beans,

Table 4

In vitro bile acid binding by steam-cooked beets (*B vulgaris*), okra (*A esculentus*), eggplant (*S malongena*), asparagus (*A officinalis*), carrots (*D carota*), green beans (*P vulgaris*), cauliflower (*B oleracea botrytis*), and turnips (*B rapa rapifera*), on equal TDF basis

Treatment	Bile acid binding ( $\mu\text{mol}/100 \text{ mg TDF}$ )	Bile acid binding relative to cholestyramine (%)
Beets	$7.61 \pm 0.08^b$	$73.3 \pm 0.7^b$
Okra	$3.80 \pm 0.07^c$	$36.7 \pm 0.7^c$
Eggplant	$3.79 \pm 0.04^c$	$36.5 \pm 0.4^c$
Asparagus	$3.56 \pm 0.06^d$	$34.3 \pm 0.6^d$
Carrots	$2.62 \pm 0.05^e$	$25.3 \pm 0.5^e$
Green beans	$2.40 \pm 0.05^f$	$23.2 \pm 0.5^f$
Cauliflower	$2.23 \pm 0.05^f$	$21.5 \pm 0.5^f$
Turnips	$0.43 \pm 0.05^g$	$4.1 \pm 0.5^g$
Cholestyramine	$10.37 \pm 0.08^a$	$100.0 \pm 0.7^a$
Cellulose	$-0.94 \pm 0.07^h$	$-9.0 \pm 0.7^h$

Means  $\pm$  SEM within a column with different superscript letters differ significantly ( $P \leq .05$ ),  $n = 6$ . The TDF (mg) used for incubation was as follows: beets, 24; okra, 44; eggplant, 38; asparagus, 29; carrots, 30; green beans, 32; cauliflower, 29; turnips, 29; cholestyramine, 25; and cellulose, 26 mg.

cauliflower, turnips. Bile acid-binding values for beets were significantly higher and those for turnips significantly lower than all the other cooked vegetables tested. Binding values for okra and eggplant were similar and significantly higher than for asparagus, carrots, green beans, and cauliflower. Values for carrots were significantly lower than those for asparagus but significantly higher than those for green beans and cauliflower.

The bile acid binding calculated for steam-cooked vegetables as eaten is given in Table 5. Bile acid binding for beets was highest (182  $\mu\text{mol}/100 \text{ g}$ , as eaten) and lowest for turnips (12  $\mu\text{mol}/100 \text{ g}$ ). Relative bile acid-binding values relative to cholestyramine were calculated based on values for beets as 17.5% (Table 3). There were significant differences in the bile acid binding of various

Table 3

In vitro bile acid binding by steam-cooked beets (*B vulgaris*), okra (*A esculentus*), eggplant (*S malongena*), asparagus (*A officinalis*), carrots (*D carota*), green beans (*P vulgaris*), cauliflower (*B oleracea botrytis*), and turnips (*B rapa rapifera*), on an equal-weight, DM basis

Treatment	Bile acid binding ( $\mu\text{mol}/100 \text{ mg DM}$ )	Bile acid binding relative to cholestyramine (%)
Beets	$1.81 \pm 0.02^b$	$17.5 \pm 0.2^b$
Okra	$1.66 \pm 0.03^c$	$16.0 \pm 0.3^c$
Eggplant	$1.43 \pm 0.01^d$	$13.7 \pm 0.1^d$
Asparagus	$1.02 \pm 0.02^e$	$9.8 \pm 0.2^e$
Carrots	$0.78 \pm 0.02^f$	$7.5 \pm 0.2^f$
Green beans	$0.76 \pm 0.02^f$	$7.3 \pm 0.2^f$
Cauliflower	$0.63 \pm 0.01^g$	$6.1 \pm 0.1^g$
Turnips	$0.12 \pm 0.01^h$	$1.2 \pm 0.1^h$
Cholestyramine	$10.37 \pm 0.08^a$	$100.0 \pm 0.7^a$
Cellulose	$-0.94 \pm 0.07^h$	$-9.0 \pm 0.7^i$

Means  $\pm$  SEM within a column with different superscript letters differ significantly ( $P \leq .05$ ),  $n = 6$ . The DM used for incubation for all the vegetables was 100 to 102 mg; cholestyramine, 24 to 25 mg; and cellulose, 24 to 27 mg.

Table 5

In vitro bile acid binding by steam-cooked beets (*B vulgaris*), okra (*A esculentus*), eggplant (*S malongena*), asparagus (*A officinalis*), carrots (*D carota*), green beans (*P vulgaris*), cauliflower (*B oleracea botrytis*), and turnips (*B rapa rapifera*), on 100-g steam-cooked, as-is basis

Treatment	Bile acid binding ( $\mu\text{mol}/100 \text{ g}$ , as is)	Calculated bile acid binding relative to cholestyramine (%)
Beets	$182.0 \pm 1.8^a$	$17.5^a$
Okra	$147.2 \pm 2.8^b$	$14.2^b$
Eggplant	$100.8 \pm 1.0^d$	$9.7^d$
Asparagus	$61.2 \pm 1.0^e$	$5.9^e$
Carrots	$108.2 \pm 2.2^c$	$10.4^c$
Green beans	$65.6 \pm 1.4^e$	$6.3^e$
Cauliflower	$46.5 \pm 1.0^f$	$4.5^f$
Turnips	$12.4 \pm 1.4^g$	$1.2^g$

Means  $\pm$  SEM within a column with different superscript letters differ significantly ( $P \leq .05$ ),  $n = 6$ . The DM used for incubation for all the vegetables was 100 to 102 mg. Calculated bile acid binding used binding data for beets as 17.5% binding relative to cholestyramine (Table 3); relative bile acid binding = ( $\mu\text{mol}/100 \text{ g as is}$ )/(17.5)/182.



steam-cooked vegetables except for asparagus and green beans, whose values were similar. Relative bile acid-binding values as eaten were, from highest to lowest, as follows: beets, okra, carrots, eggplant, green beans, asparagus, cauliflower, turnips.

#### 4. Discussion

Steam cooking resulted in an increase in TDF in okra, carrots, and turnips, whereas it decreased in eggplant, asparagus, green beans, and cauliflower. The change in TDF appears to be related to the availability of carbohydrate content influenced by cooking. There was 3% increase in protein content by cooking these vegetables, which may be due to redistribution of relative proportions of nutrients. Cooking resulted in loss of minerals and lipid in all the vegetables except for cauliflower, where values increased. Comparison of raw vs steam-cooked vegetables suggested that there was an increase in protein content, variable effect on TDF, and a loss in lipid and mineral contents.

Cholestyramine bound 90% of the bile acids. These values are similar to the previously reported observations [16,37]. Story and Kritchevsky [38] reported 81% bile acid binding by cholestyramine using 50 mg of substrate and 50  $\mu$ mol of bile acids. Higher bile acid binding by cholestyramine in our studies may be due to the use of physiologic pH and/or a higher substrate-to-bile acid ratio. Previously reported bile acid binding by uncooked vegetables was as follows: okra, 16%; beets, 11%; asparagus, 4%; and only 1% for eggplant, carrots, green beans, cauliflower, and turnips [29]. Most of these vegetables are consumed after cooking. Significantly higher bile acid binding by beets, asparagus, carrots, green beans, and cauliflower by steam cooking compared with their uncooked binding values suggests that these vegetables are more healthful after steam cooking. The differences in bile acid binding between various vegetables tested may relate to their phytonutrients (antioxidants, chlorophyll, flavonoids, glucosinolates, hydroxycinnamic acid, isothiocyanates, micro elements, and tannins), hydrophobicity, or active binding sites. On a DM basis, the 6% to 18% relative bile acid binding by steam-cooked vegetables tested, except for turnips, indicates their high healthful potential. Relative bile acid binding of 5% to 9% for oat bran, oat-bran ready-to-eat cereals, and barley fractions (cereals with US Food and Drug Administration approval for label health claim for lowering cholesterol) have been reported [16,25,26]. Data suggest that vegetables tested herein could qualify for a US Food and Drug Administration label health claim for lowering cholesterol. How other cooking methods influence the bile acid binding of vegetables needs to be evaluated.

Bile acid binding of oat bran; whole barley flour; and  $\beta$ -glucan enriched barley fractions [25], ready to eat cereals [26], Bengal gram, black eye bean, black gram, kidney bean, lima bean, moth bean, and soybean [27,28] have also been related to their DM content. Evaluating healthful properties

(cholesterol lowering and excretion of toxic metabolites) of various vegetables and food fractions as they are normally consumed would be desirable by testing their bile acid binding on a DM basis.

Previously Kahlon et al [29] reported the following values for bile acid binding on a TDF basis: uncooked okra, 49%; beets, 50%; asparagus, 12%; eggplant, 3%; turnips, 5%; green beans, 3%; carrots, 3%; and cauliflower, 2%. Cooking okra, carrots, and turnips resulted in significant increases in their TDF content; however this additional TDF resulted in lower, higher, and same bile acid binding, respectively, compared with raw vegetable binding values. Data suggest that there was no consistent trend between TDF content of the vegetables tested and their bile acid-binding values. Significantly higher bile acid for cooked beets, eggplant, asparagus, carrots, green beans, and cauliflower compared with those reported for these vegetables uncooked is very encouraging because these vegetables are normally consumed after cooking. Data suggest that steam-cooked vegetable are more healthful than those consumed raw (uncooked). There was a similar amount (29–30 mg) of TDF used per incubation for asparagus, carrots, cauliflower, and turnips; however, their bile acid binding on a TDF basis varied significantly. Data suggest that bile acid binding is not related to the TDF content of the cooked vegetables tested herein. This is in agreement with previous reports that bile acid binding of various dry beans was not related to the TDF content [27,28].

That bile acid-binding values for beets and okra (100 g, as-eaten basis) were significantly higher than all the other vegetables tested suggest that these 2 vegetables have good health-promoting potential. Carrots contained highest amount of DM (13.8%); however, its binding values were significantly lower (37%–68%) than those of okra and beets. The highest bile acid binding for beets is very encouraging; how much of its binding may be related to its betanin content will be evaluated in subsequent studies. Data suggest that bile acid-binding potential appears to be related to unique phytonutrients, which will be determined in future investigations.

In conclusion, relative to cholestyramine, the *in vitro* bile acid binding on DM basis was, for beets, 18%; okra, 16%; eggplant, 14%; asparagus, 10%; carrots, 8%; green beans, 7%; cauliflower, 6%; and turnips, 1%. These results point to the significant differences in health-promoting potential of steam-cooked vegetables (from highest to lowest, beets, okra, eggplant, asparagus, carrots and green beans, cauliflower, turnips) as indicated by their bile acid binding on a DM basis. Compared with raw vegetables, steam cooking resulted in significantly higher bile acid binding by beets, eggplant, asparagus, carrots, green beans, and cauliflower. Data suggest that the healthful potential of these vegetables is significantly increased by steam cooking. Because bile acid binding was determined on DM obtained after drying cooked vegetables for 48 hours at 68°C to a constant weight, any effect on bile acid binding due to prolonged drying time

would be evaluated in subsequent investigations. Inclusion of steam-cooked beets, okra, eggplant, asparagus, carrots, green beans, and cauliflower in our daily diet as healthful vegetables should be encouraged. These vegetables, when consumed regularly, may lower the risk of premature degenerative diseases (heart disease and cancer), improve public health, and advance human nutrition research.

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